

# Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons

Jiangyan Yang<sup>a,1</sup>, Evelyne Ruchti<sup>a,b,c,1</sup>, Jean-Marie Petit<sup>a,c</sup>, Pascal Jourdain<sup>a,c</sup>, Gabriele Grenningloh<sup>a</sup>, Igor Allaman<sup>a,2,3</sup>, and Pierre J. Magistretti<sup>b,c,a,2,3</sup>

<sup>a</sup>Laboratory of Neuroenergetics and Cellular Dynamics, Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland; <sup>b</sup>Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia; and <sup>c</sup>Centre de Neurosciences Psychiatriques, Département de Psychiatrie, Centre Hospitalier Universitaire Vaudois (CHUV), Site de Cery, CH-1008 Prilly/Lausanne, Switzerland

Edited by Marcus E. Raichle, Washington University in St. Louis, St. Louis, MO, and approved June 27, 2014 (received for review December 19, 2013)

L-lactate is a product of aerobic glycolysis that can be used by neurons as an energy substrate. Here we report that in neurons L-lactate stimulates the expression of synaptic plasticity-related genes such as *Arc*, *c-Fos*, and *Zif268* through a mechanism involving NMDA receptor activity and its downstream signaling cascade Erk1/2. L-lactate potentiates NMDA receptor-mediated currents and the ensuing increase in intracellular calcium. In parallel to this, L-lactate increases intracellular levels of NADH, thereby modulating the redox state of neurons. NADH mimics all of the effects of L-lactate on NMDA signaling, pointing to NADH increase as a primary mediator of L-lactate effects. The induction of plasticity genes is observed both in mouse primary neurons in culture and in vivo in the mouse sensory-motor cortex. These results provide insights for the understanding of the molecular mechanisms underlying the critical role of astrocyte-derived L-lactate in long-term memory and long-term potentiation in vivo. This set of data reveals a previously unidentified action of L-lactate as a signaling molecule for neuronal plasticity.

brain energy metabolism | learning and memory | astrocyte–neuron interaction | astrocyte–neuron lactate shuttle

The transfer of L-lactate from astrocytes to neurons was recently shown to be necessary for the establishment of long-term memory (LTM) in an inhibitory avoidance (IA) paradigm and for the maintenance of in vivo long-term potentiation (LTP) in the rodent hippocampus (1). This key role of L-lactate in neuronal plasticity mechanisms was demonstrated in experiments in which specific pharmacological and gene expression down-regulation interventions were implemented to prevent the production of L-lactate from glycogen—which is exclusively localized in astrocytes—and its release from these cells in the hippocampus during behavioral training (1). Such interventions completely prevented the establishment of LTM and their effect was fully reversed by the intrahippocampal administration of L-lactate during the training session. The fact that glucose at equicaloric concentrations only marginally mimicked the rescuing effect of L-lactate was taken as an unexpected indication that the primary mechanism of action of L-lactate on plasticity mechanisms was independent of its ability to act as an energy substrate. A role of L-lactate in memory processes was also recently shown in other behavioral paradigms (2, 3). We therefore set out to investigate the molecular mechanisms at the basis of the function of L-lactate on neuronal plasticity.

Molecular mechanisms underlying both LTM and long-term plasticity include the induction of expression of a group of immediate early genes (IEGs) such as early growth response 1 (*Zif268* or *Egr1*), CCAAT/enhancer binding protein (*C/EBP*), and proto-oncogene *c-Fos* (*c-Fos*) as well as activity-regulated cytoskeletal-associated protein (*Arc* or *Arg3.1*) as a direct effector protein at the synapse, which all participate to different physiological processes associated with neuronal plasticity (4–6). Although stimulation of expression of these IEGs is not

restricted to plasticity processes, they are considered as key plasticity-related genes in sustaining such phenomena. In addition, late response genes such as brain-derived neurotrophic factor (BDNF) have also been demonstrated to be major intermediates of plasticity-related processes (7). A role of NMDA receptors (NMDARs) in such plasticity mechanisms is well-established (5, 8).

In this article we describe a cascade of molecular events demonstrating that L-lactate stimulates plasticity-related gene expression in neurons through modulation of NMDAR activity associated with changes in redox cellular state. The induction of plasticity gene expression by L-lactate was observed in primary cultures of neurons as well as in vivo in the sensory-motor cortex of mice.

## Results

**L-Lactate Induces the Expression of Plasticity Genes.** L-lactate applied to primary neuronal cultures of the mouse neocortex stimulated *Arc* expression in a concentration- and time-dependent manner (Fig. 1*A* and *B*). Significant effects started to be observed at 2.5 mM ( $48.2 \pm 12.1\%$  increase compared with control values), increased up to 20 mM, and are maximally expressed after 1 h of treatment. L-lactate induced the expression of two other synaptic plasticity-related IEGs, *c-Fos* and *Zif268* (4–6) (Fig. 1*B*). The effect

## Significance

The transfer of lactate, a product of aerobic glycolysis, from astrocytes to neurons was recently shown to be necessary for the establishment of long-term memory and for the maintenance of in vivo long-term potentiation. Here, we report that lactate induces the expression of plasticity genes such as *Arc*, *c-Fos*, and *Zif268* in neurons. The action of lactate is mediated by the modulation of NMDA receptor activity and the downstream Erk1/2 signaling cascade, through a mechanism associated with changes in the cellular redox state. These observations unveil an unexpected role of lactate as a signaling molecule in addition to its role in energy metabolism and open a previously unidentified research avenue for the study of neuronal plasticity and memory.

Author contributions: G.G., I.A., and P.J.M. designed research; J.Y., E.R., J.-M.P., and P.J. performed research; J.Y., E.R., J.-M.P., P.J., G.G., I.A., and P.J.M. analyzed data; and I.A. and P.J.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

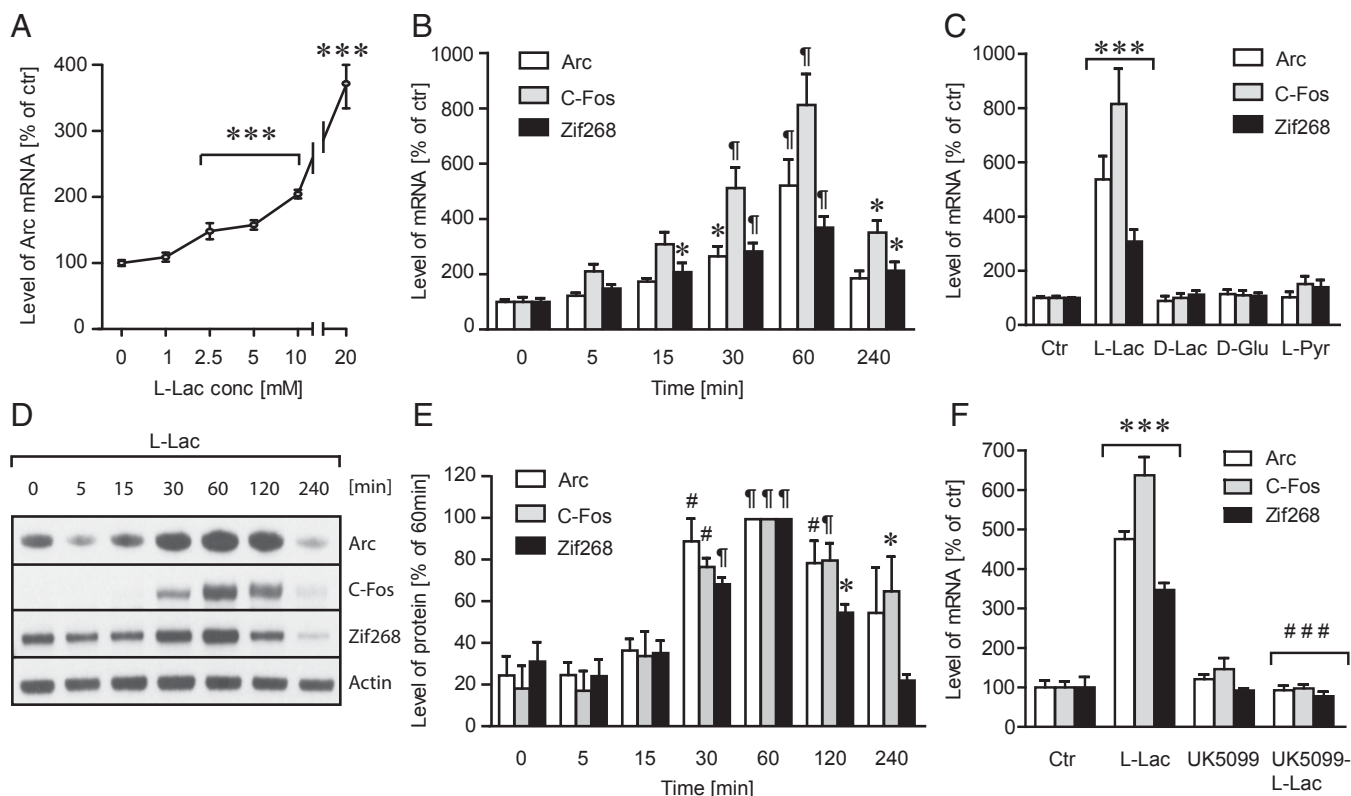
Freely available online through the PNAS open access option.

<sup>1</sup>J.Y. and E.R. contributed equally to this work.

<sup>2</sup>I.A. and P.J.M. contributed equally to this work.

<sup>3</sup>To whom correspondence may be addressed. Email: igor.allaman@epfl.ch or pierre.magistretti@kaust.edu.sa.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1322912111/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1322912111/-DCSupplemental).



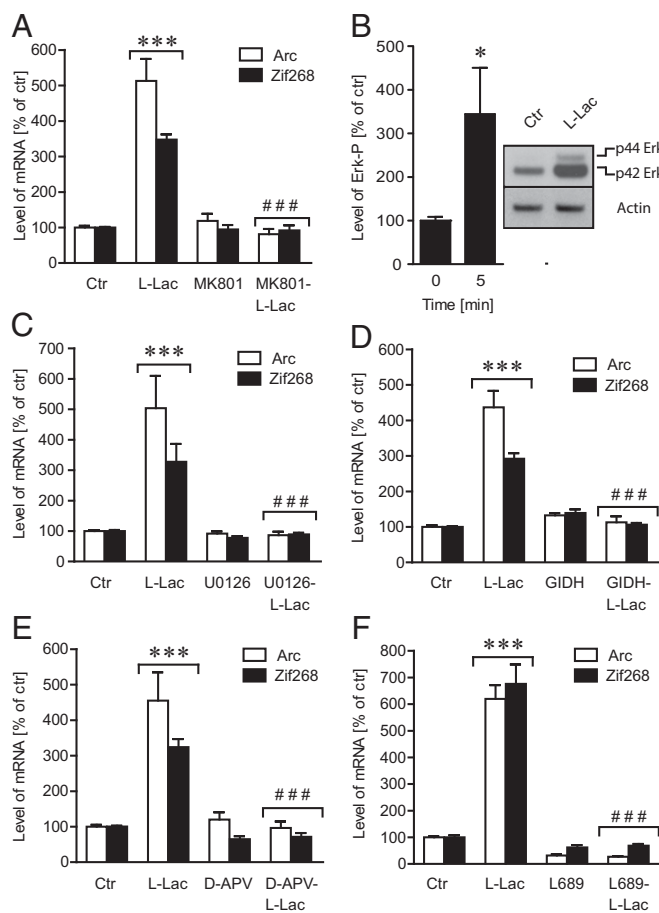
**Fig. 1.** L-lactate stimulates the expression of the IEGs Arc, c-Fos, and Zif268 in cultured neurons. (A) Concentration–response curve of Arc mRNA expression following exposure to L-lactate (L-Lac) ( $n = 9$ , three independent experiments). (B) Time course of Arc, c-Fos, and Zif268 mRNA induction by 20 mM L-lactate ( $n = 9$ , three independent experiments). (C) IEG mRNA expression 1 h following exposure to various energy substrates applied at 20 mM for L-Lac, D-lactate (D-Lac), L-pyruvate (L-Pyr), or 10 mM for D-glucose (D-Glu) ( $n = 12$ , four independent experiments). (D) Western blots of time-course analysis of IEG protein expression following exposure to 20 mM L-lactate. (E) Quantification of Western blot bands shown in D ( $n = 6$ –5, five independent experiments). (F) IEG mRNA expression following 1-h exposure to 20 mM L-lactate in the presence or absence of the MCT inhibitor UK5099 (50  $\mu$ M) ( $n = 9$ , three independent experiments). Data in A–C, E, and F are expressed as percentage of respective control (Ctr or 60-min) values and are mean  $\pm$  SEM. \*\*\* $P \leq 0.001$ , \* $P \leq 0.01$ , and \* $P \leq 0.05$  vs. respective control and \*\*\*\* $P \leq 0.001$  vs. respective L-lactate condition with ANOVA followed by Dunnett's [A (0–10 mM) and B] or Bonferroni's (C, E, and F) post hoc tests, or unpaired  $t$  test [A (0 and 20 mM)].

of L-lactate on expression of IEGs was specific; D-lactate (non-metabolized enantiomer), L-pyruvate (another monocarboxylate), and D-glucose (used at a similar or equicaloric concentration to 20 mM L-lactate) were inactive under identical experimental conditions (Fig. 1C). Increased IEG mRNA expression levels induced by L-lactate were correlated with the protein levels as shown by Western blot experiments (Fig. 1D), with maximum values being observed after 1 h of treatment (fold increase: 4.0, 5.5, and 3.2 vs. control values for Arc, c-Fos, and Zif268, respectively; Fig. 1E). To assess the importance of intracellular L-lactate transport on IEG mRNA expression, the monocarboxylate transporters (MCTs) inhibitor UK5099 was used. As shown in Fig. 1F, the stimulatory effects of L-lactate on IEG mRNA expression was fully prevented in the presence of 50  $\mu$ M UK5099. In addition to IEGs, expression levels of *BDNF*, a key mediator of late-phase synaptic plasticity processes (7), was also significantly induced by 20 mM L-lactate at later time points, with maximum values obtained at 4 h ( $351.0 \pm 58.9\%$  of increase compared with control values; mean  $\pm$  SEM;  $n = 9$ , three independent experiments; Student  $t$  test:  $P \leq 0.001$ ). In contrast to Arc, c-Fos and Zif268 mRNA expression the IEG *C/EBP $\gamma$*  isoform, that has been shown to participate in synaptic plasticity and memory formation processes (4) was not modulated by L-lactate (20 mM) after 1 h of treatment ( $100.0 \pm 2.4\%$  compared with control values; mean  $\pm$  SEM;  $n = 6$ , two independent experiments,  $P > 0.05$  by Student  $t$  test).

**L-Lactate Potentiates NMDAR Signaling.** NMDARs are ionotropic glutamate receptors that are prototypical mediators of synaptic plasticity processes involving the induction of plasticity-related genes such as Arc and Zif268 (5, 8). We therefore considered the possibility that NMDARs could be potential candidates as mediators of the effects of L-lactate on IEG expression. Consistent with such involvement, L-lactate-induced Arc and Zif268 expression was completely abolished in the presence of the NMDAR antagonist MK801 at 40  $\mu$ M at both mRNA (Fig. 2A) and protein levels (Fig. S1A and H).

L-lactate also increased the phosphorylation of the Extracellular signal-regulated kinase 1/2 (Erk1/2, also known as p44/p42 Mitogen-activated protein kinase, p44/p42 MAPK), involved in a key signaling cascade mediating the NMDAR-dependent neuronal plasticity (8–11) (Fig. 2B). Increased Erk1/2 phosphorylation occurred within 5 min following L-lactate application (3.4-fold increase vs. control values, Fig. 2B). Consistent with an involvement of the Erk1/2 pathway in the action of L-lactate, stimulation of Arc and Zif268 mRNA levels (Fig. 2C) and protein levels (Fig. S1B and H) was blocked by the Erk1/2 kinase inhibitor U0126 (10  $\mu$ M). Moreover, phosphorylation of Erk1/2 induced by L-lactate was selective because it was not reproduced by L-pyruvate (Fig. S1I). This set of results clearly identifies NMDARs and the downstream Erk1/2 signaling cascade as main transducers of L-lactate effects.

NMDAR activity is under the control of complex modulations (12, 13). In particular, NMDARs possess two main regulatory binding domains that are referred to as the glutamate and the



**Fig. 2.** NMDAR activation and Erk1/2 signaling mediate the effects of L-lactate. (A) Arc and Zif268 mRNA expression 1 h following exposure to 20 mM L-lactate in the presence or absence of MK801 (40  $\mu$ M) ( $n = 9$ –12, three or four independent experiments). (B) Representative Western blot and its quantification for Erk1/2 phosphorylation after a 5-min application of 20 mM L-lactate ( $n = 11$ , four independent experiments). (C–F) Arc and Zif268 mRNA expression following 1-h exposure to 20 mM L-lactate in the presence or absence of (C) the Erk1/2 inhibitor U0126 (10  $\mu$ M) ( $n = 9$ , three independent experiments), (D) glutamate dehydrogenase (GIDH, 6.75 U/mL) ( $n = 6$ , two independent experiments), (E) the NMDAR glutamate site antagonist D-APV (50  $\mu$ M) ( $n = 9$ , three independent experiments), or (F) the NMDAR glycine site antagonist L-689,560 (L689, 10  $\mu$ M) ( $n = 9$ , three independent experiments). All data are expressed as percentage of respective control (Ctrl or 0-min) values as mean  $\pm$  SEM. \*\*\* $P \leq 0.001$ , \* $P \leq 0.05$  vs. respective Ctrl and ### $P \leq 0.001$  vs. respective L-lactate condition with ANOVA followed by Bonferroni's post hoc test, or unpaired  $t$  test (B).

coagonist glycine sites, respectively (13). Efficient opening of NMDARs usually requires binding of agonists to both regulatory sites. Using two different approaches, we show that glutamate binding was necessary for L-lactate to produce its effects. Indeed, in situ degradation of glutamate, through the addition of glutamate dehydrogenase (GIDH, 6.75 U/mL), as well as application of the glutamate binding site selective competitive inhibitor D-(-)-2-amino-5-phosphonopentanoic acid (D-APV) (50  $\mu$ M), suppressed the effect of L-lactate on Arc and Zif268 expression (Fig. 2D and E). In addition, L-689,560 (10  $\mu$ M) a selective antagonist at the glycine site, completely antagonized the stimulatory effect of L-lactate on IEG mRNA expression (Fig. 2F). A similar effect was obtained using another glycine site antagonist, 5,7-dichlorokynurenic acid (DCKA, 4 mM) (Fig. S1C). The inhibitory effects of GIDH, D-APV, L-689,560, and DCKA were confirmed at the protein levels (Fig. S1D–G).

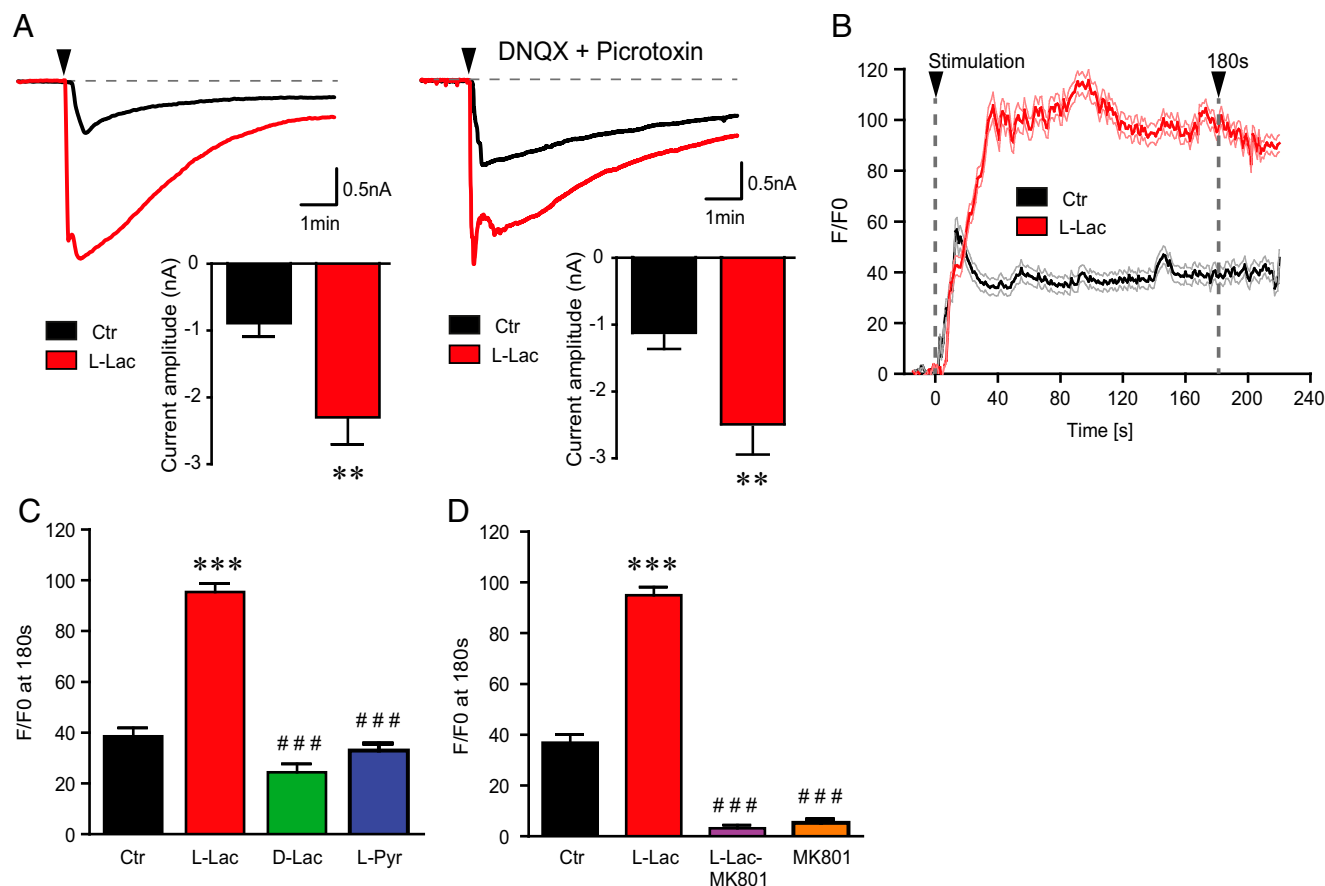
In agreement with the activation of NMDARs by L-lactate, electrophysiological recordings of neurons demonstrate that NMDAR activation is potentiated by L-lactate. In initial experiments 20 mM L-lactate was used; however, a concentration of 10 mM L-lactate was used in the subsequent electrophysiological and calcium imaging studies. As shown in Fig. 3A, Left, L-lactate enhanced the inward current evoked by application of glutamate (0.5  $\mu$ M) and glycine (200  $\mu$ M) with a peak value of  $-2.30 \pm 0.40$  nA in the presence of L-lactate ( $n = 8$ , eight independent experiments) compared with a peak value of  $-0.89 \pm 0.17$  nA ( $n = 10$ , 10 independent experiments) in the control condition. This increase was completely abolished in the presence of 40  $\mu$ M MK801 ( $-0.33 \pm 0.07$  nA MK801 vs.  $0.37 \pm 0.04$  nA for MK801 + L-lactate;  $n = 5$ –6, five or six independent experiments,  $P > 0.05$  by Student  $t$  test). To further demonstrate that this L-lactate-dependent inward current potentiation is purely linked to the activation of NMDARs a set of experiments was performed in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 40  $\mu$ M), a specific antagonist of AMPA receptors, and picrotoxin (30  $\mu$ M), an antagonist of GABA<sub>A</sub> receptors. Results clearly showed that the potentiation by L-lactate of the inward current evoked by glutamate and glycine persisted in the presence of both inhibitors [peak value of  $-2.45 \pm 0.44$  nA in the presence of L-lactate ( $n = 8$ , eight independent experiments) compared with a peak value of  $-1.10 \pm 0.23$  nA ( $n = 8$ , eight independent experiments) in the control condition (Fig. 3A, Right)].

Opening of ionotropic NMDARs results in calcium influx, leading to an increase in intracellular calcium ( $[Ca^{2+}]_i$ ) (8). Accordingly, potentiation of NMDARs by L-lactate, as demonstrated by electrophysiological recordings, was associated with NMDAR-dependent  $[Ca^{2+}]_i$  increase. Fig. 3B shows modulation in  $[Ca^{2+}]_i$  monitored by calcium imaging that is induced by L-lactate (10 mM) following NMDAR activation by glutamate and glycine, compared with a control condition. The increase was rapid (peak at  $32.27 \pm 4.81$  s,  $n = 11$ , 11 independent experiments) and maintained over time up to 240 s. This effect was specific for L-lactate (about 2.5-fold increase) and was not reproduced by D-lactate or L-pyruvate (Fig. 3C). The L-lactate-induced calcium influx was confirmed to be NMDAR-dependent because it is prevented in the presence of MK801 (Fig. 3D), D-APV, and L-689,560 (Fig. S2A and B). Furthermore, in the absence of calcium in the extracellular medium L-lactate did not affect intracellular calcium levels (Fig. S2C).

**NADH Mimics the Effects of L-Lactate.** Exposure of neurons to L-lactate modified the intracellular NADH/NAD ratio (Fig. 4A) as a result of the metabolic processing of L-lactate to L-pyruvate under normoxic conditions. Consistent with this, L-pyruvate did not affect the redox state of neurons (Fig. 4A). Moreover, direct application of NADH (4 mM) resulted in the stimulation of IEG expression at both mRNA and protein levels as well as Erk1/2 phosphorylation to an extent similar to that induced by L-lactate (Fig. 4B–D). Importantly, the effects of NADH on IEG expression and Erk1/2 phosphorylation were antagonized by MK801 (40  $\mu$ M) (Fig. 4B–D). NADH application also resulted in increases in intracellular calcium levels following NMDAR activation by glutamate and glycine in an MK801-sensitive manner (Fig. 4E). These results further strengthen the relationship between redox state changes evoked by L-lactate, NMDAR activation, and IEG expression.

**L-Lactate Induces IEG Expression in Vivo.** Direct intracortical injections of L-lactate (four injections of 0.5  $\mu$ L of 10 mM solution) in the sensory-motor cortex of anesthetized adult mice also resulted within 1 h of application in a significant increase in Arc, c-Fos, and Zif268 mRNA expression (by  $61 \pm 13\%$ ,  $60 \pm 12\%$ , and  $46 \pm 8\%$ , respectively) compared with D-lactate (10 mM) injected in contralateral areas (Fig. 5A). L-pyruvate (10 mM) was similarly ineffective (Fig. 5B). Of note, IEG expression levels in D-lactate- and L-pyruvate-injected cortices were not higher than those





**Fig. 3.** L-lactate potentiates NMDA-receptor inward currents and the ensuing increase in intracellular calcium. (A, Left) Representative electrophysiological current traces recorded from two different patched neurons, illustrating typical currents triggered by glutamate (0.5  $\mu$ M) and glycine (200  $\mu$ M) in the absence (Ctr, black trace) or the presence of 10 mM L-lactate (L-Lac, red trace). Arrowhead indicates the time of drug application. The bar chart shows quantification of the difference in amplitude for the two specific currents ( $n = 8-10$ , 8–10 independent experiments). (Right) Same as Left but in the presence of 40  $\mu$ M DNQX and 30  $\mu$ M picrotoxin in the medium. The bar chart shows quantification of the difference in amplitude for the two specific currents ( $n = 8$ , eight independent experiments). (B) Representative average fluctuation curve of  $\text{Ca}^{2+}$  fluorescence intensity as measured by calcium imaging with Fluo-4 AM of recorded neurons following stimulation of NMDARs. Same Ctr and L-Lac conditions as in A, Left. Mean (in dark color)  $\pm$  SEM (in light color) of 245 and 235 for Ctr and L-lactate conditions, respectively, recorded neurons from four independent experiments. Arrowhead indicates the time of drug application. (C and D) Average changes of  $\text{Ca}^{2+}$  fluorescence intensity of recorded neurons after 180 s of glutamate (0.5  $\mu$ M) and glycine (200  $\mu$ M) stimulation (Ctr) in the presence of L-lactate, D-lactate, or L-pyruvate (10 mM) ( $n = 231-287$ , four independent experiments) (C) or in the presence of 10 mM L-lactate and/or MK801 (40  $\mu$ M) ( $n = 199-261$ , four independent experiments) (D). All data are means  $\pm$  SEM. \*\*\* $P \leq 0.001$ , \*\* $P \leq 0.005$  vs. respective Ctr and ### $P \leq 0.001$  vs. respective L-lactate condition with ANOVA followed by Bonferroni's post hoc test (C and D) or unpaired  $t$  test (A).

measured in control, noninjected, more posterior cortical samples, demonstrating the absence of stimulatory effects on IEG expression in response to surgery or to D-lactate and L-pyruvate administration (Fig. S3).

## Discussion

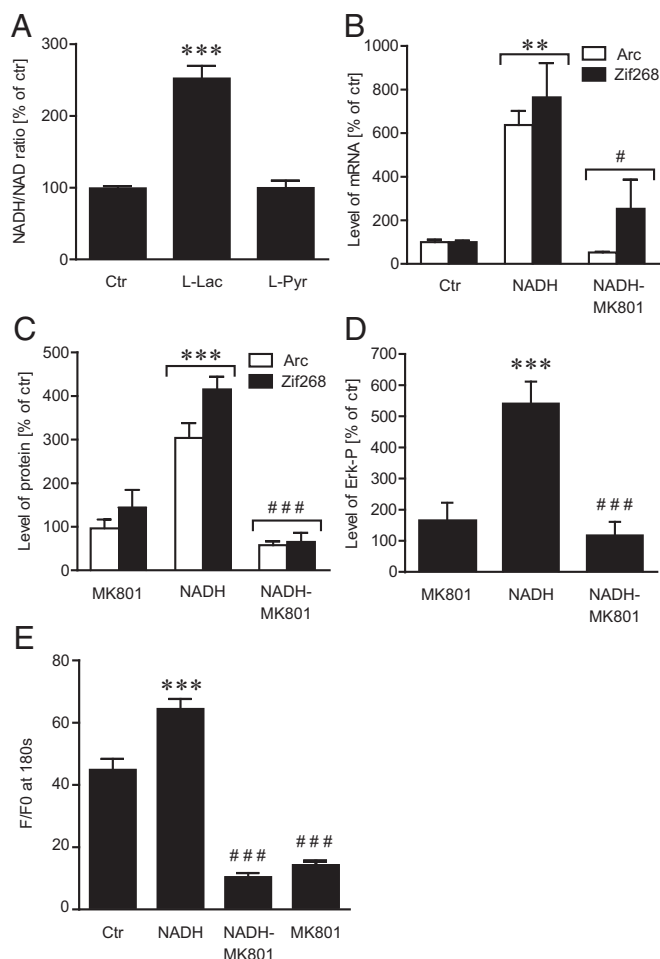
Transfer of lactate from astrocytes to neurons is required for LTM (1), but the underlying molecular mechanisms of this effect are unknown. Here, we describe a cascade of molecular events showing that L-lactate stimulates plasticity-related gene expression in neurons through a modulation of NMDAR activity that is associated with changes in redox cellular state.

**Effects of L-Lactate on Plasticity-Related Gene Expression.** Previous observations reported the ability of L-lactate to stimulate gene expression in different cell types in culture such as L6 cells, vasculogenic stem cells, mesenchymal stem cells, or fibroblasts although the activation of specific transcription factors (14–17). In the present study we show that L-lactate induces the expression of key plasticity-related genes, namely, *Arc*, *Zif268*, *c-Fos*, and *BDNF* in neuronal cultures. This effect is selective and it

is not reproduced by its nonmetabolized enantiomer D-lactate or by the energy substrates L-pyruvate and D-glucose applied at equicaloric concentrations. The dose–response curve on *Arc* mRNA expression demonstrates that L-lactate is significantly effective at a concentration of 2.5 mM. This active concentration is in the range of estimated extracellular L-lactate concentrations measured in rodent and human brains [ranging from 0.5 to 5 mM (18)] and is therefore physiologically relevant.

Consistent with these observations, we show that the stimulatory effect of L-lactate on *Arc*, *c-Fos*, and *Zif268* mRNA expression also occurs in vivo, an effect not reproduced by D-lactate or L-pyruvate.

**Involvement of NMDARs.** NMDARs are glutamate-gated ion channels pivotal in the regulation of synaptic plasticity processes associated with LTM in different learning paradigms (6). They play a role in synaptogenesis, experience-dependent synaptic remodeling, and long-lasting changes in synaptic efficacy such as LTP (6, 13, 19). NMDARs control the expression of plasticity-related IEG genes, including *Arc*, *c-Fos*, and *Zif268*. This process is mediated by intracellular  $\text{Ca}^{2+}$  elevation and subsequent activation of the Erk1/2 signaling pathways (8, 10, 11).



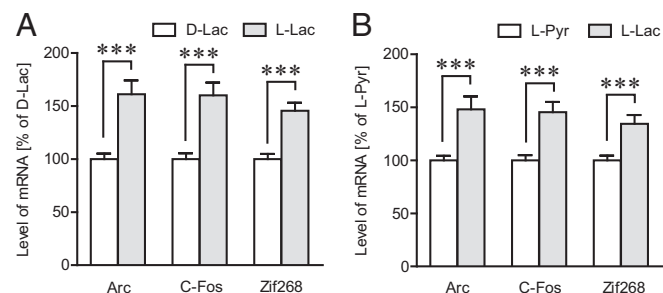
**Fig. 4.** Role of redox state in the effects of L-lactate. (A) NADH/NAD ratio 10 min after application of 20 mM L-lactate or L-pyruvate ( $n = 12$ , four independent experiments). (B–D) Effect of 4 mM NADH (1 h), in the presence or absence of MK801 (40  $\mu$ M), on the levels of expression of Arc and Zif268 mRNA ( $n = 11$ , four independent experiments) (B) and protein ( $n = 5$ , five independent experiments) (C) and on Erk1/2 phosphorylation ( $n = 5$ , five independent experiments) (D). (E) Average changes of  $\text{Ca}^{2+}$  fluorescence intensity of recorded neurons after 180 s of glutamate (0.5  $\mu$ M) and glycine (200  $\mu$ M) stimulation (Ctrl) in the presence of NADH (4 mM) and/or MK801 (40  $\mu$ M) ( $n = 109$ –215, four independent experiments). All data are means  $\pm$  SEM. \*\*\* $P \leq 0.01$ , \*\*\*\* $P \leq 0.001$ , vs. respective Ctrl (A, B, and E) and MK801 (C and D) conditions and # $P \leq 0.05$ , ### $P \leq 0.001$  vs. respective NADH condition with ANOVA followed by Bonferroni's post hoc test.

Experimental *in vitro* evidence obtained in the present study reveals that NMDARs and their downstream Erk1/2 signaling cascade mediate the effects of L-lactate. Thus, (i) L-lactate increases Erk1/2 phosphorylation and (ii) blockade of NMDAR activity and/or Erk1/2 phosphorylation by specific inhibitors (MK801 and U0126, respectively) abolishes L-lactate-induced IEG expression.

**L-Lactate Potentiates NMDA Activity.** NMDARs are tetrameric protein complexes composed of a combination of glycine-binding NR1 subunits and glutamate-binding NR2 subunits and are under the control of multiple regulatory mechanisms (13). Activation of NMDARs requires the binding of both the agonist (glutamate) and a coagonist (glycine or D-serine). In line with this, we observed that selective inhibition of both binding sites (using L-689,560 and D-APV, inhibitors of the glycine and glutamate binding sites, respectively) as well as *in situ* degradation of glutamate by glutamate dehydrogenase impairs the ability of L-lactate to induce IEG

expression. The set of results reported in this study indicates that the effects of L-lactate do not result from the activation of silent NMDARs but rather that L-lactate acts as a modulator of already activated (by glutamate and glycine site coagonists) NMDARs. As a matter of fact, L-lactate potentiated the NMDAR-dependent inward current and calcium influx evoked by application of both glutamate and glycine. Consistent with the effect of L-lactate on IEG expression, potentiation of NMDARs is selective for L-lactate; NMDAR-dependent inward current or calcium influx evoked by application of both glutamate and glycine were not potentiated by D-glucose (Fig. S4), D-lactate, or L-pyruvate. Although the experimental evidence presented provides the first evidence to our knowledge that NMDA receptor-mediated signaling is positively modulated by L-lactate, the cellular sites of action (pre/postsynaptic) and the precise molecular determinants possibly involved in addition to changes in the redox state will require further investigations.

**The Effects of L-Lactate Are Associated with Intracellular Redox Changes.** L-lactate transport into cells through MCTs and further metabolism seems an obligatory route to affect cell energetics and functions, although recent evidence suggests that it could also affect cell responsiveness through its binding to a specific G-coupled cell surface receptor, GPR81 (20). Consistent with a transport-mediated effect of L-lactate, IEG expression was blocked in the presence of the MCT inhibitor UK5099. Once inside cells, L-lactate is metabolized to L-pyruvate by L-lactate dehydrogenase, which, using NAD<sup>+</sup> as a cofactor, results in the production of reducing equivalents in the form of NADH. NADH production is therefore the only specific process that differentiates L-lactate and L-pyruvate metabolism. As observed here, L-lactate (but not L-pyruvate) metabolism gives rise to an intracellular increase of the NADH/NAD ratio, hence modifying the intracellular neuronal redox state. Redox-sensitive NMDA regulatory sites are present on the NR1 subunit, which favors NMDAR activity when in a reduced state and decreases its activity when oxidized, as demonstrated in particular with the use of DTT and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) (21, 22). Interestingly, we observed that the stimulatory effect of L-lactate on IEG expression is prevented in the presence of the oxidizing agent DTNB (Fig. S5). Further supporting a role of changes in the redox state of neurons in the modulatory effects of L-lactate on NMDAR-mediated signaling, we observed stimulatory effects of NADH on intracellular calcium increases evoked by application of both glutamate and glycine, Erk1/2 phosphorylation, and IEG expression that are markedly inhibited in the presence of MK801. Overall, results presented in this article point to a redox state change as the likely mediator of L-lactate.



**Fig. 5.** L-lactate stimulates the expression of the IEGs Arc, c-Fos, and Zif268 *in vivo*. (A and B) IEG mRNA levels following injection of 10 mM L-lactate [and D-lactate (A) or L-pyruvate (B) in contralateral areas] in sensory-motor cortex of mice ( $n = 7$ –8 mice). Data in A and B are expressed as percentage of respective control values and are means  $\pm$  SEM. \*\*\* $P \leq 0.001$  vs. respective control with ANOVA followed by Bonferroni's post hoc test.

## Role of Astrocytes in L-Lactate Production and Neuronal Plasticity.

Astrocytes are emerging as having significant roles in several homeostatic processes in the brain (23–26). In particular, astrocytes mediate activity-driven energy delivery to neurons in the form of L-lactate (25, 27–30). L-lactate can be produced and released from astrocytes through two neuronally dependent signaling mechanisms. First, through glutamate-evoked glycolysis, a mechanism also known as the astrocyte–neuron lactate shuttle, glutamate uptake and the associated increase in intracellular sodium concentration activate the  $\alpha 2$  subunit of the Na/K-ATPase and promote glucose uptake and L-lactate production (28, 29). A similar neuronal activity-dependent mechanism has been recently shown for potassium as an activator of astrocytic glycolysis (31). Another mechanism for L-lactate production is glycogenolysis (32). In the brain, glycogen is only contained in astrocytes, where glycogenolysis can be evoked by noradrenaline and vasoactive intestinal peptide (33). The importance of L-lactate transfer from astrocytes to neurons to sustain cognitive functions has been shown (1–3). In particular, a major role of glycogen-derived L-lactate in LTM and LTP in a hippocampal-based cognitive process such as IA has been demonstrated (1). The effects of L-lactate are associated with molecular changes required for memory formation such as Arc induction as well as CREB and cofilin phosphorylation (1). Hence, the present results showing that L-lactate (unlike D-glucose and L-pyruvate) induces synaptic plasticity gene expression (i.e., *Arc*, *Zif268*, and *c-Fos*) both in vitro and in vivo are fully consistent with such observations. Control of L-lactate metabolism in astrocytes as operated by both glutamate- and potassium-mediated glycolysis (28, 31) and by monoamine-mediated glycogenolysis (33) is likely to add another level of regulation of NMDA activity in general and NMDAR-mediated synaptic plasticity processes in particular.

It has previously been reported that L-lactate can modulate neuronal excitability through ATP-dependent mechanisms

involving modulation of KATP channels (ref. 25 and references therein). Very recently, neuronal activity has been shown to be also modulated through extracellular actions of L-lactate involving GPR81 or yet-unknown mechanisms (34, 35). Here, we report that L-lactate stimulates synaptic plasticity-related gene expression in neurons by a mechanism associated with changes of the intracellular redox state and the modulation of the NMDAR activity and its downstream signaling cascade Erk1/2. These results provide important clues for understanding the molecular bases underlying the critical role played by astrocyte-derived L-lactate in the establishment of LTM. More generally, they shed new light on the role played by L-lactate and by astrocyte–neuron interactions in the regulation of synaptic plasticity processes, and hence in learning and memory.

## Materials and Methods

Experiments were conducted in accordance with the Swiss Federal Guidelines for Animal Experimentation and were approved by the Cantonal Veterinary Office for Animal Experimentation (Vaud, Switzerland). Primary cultures of cortical neurons (days in vitro 14–21) were treated by direct application of compounds into the culture medium, or incubation medium, using 50–100 $\times$  stock solutions of L-lactate, D-lactate, L-pyruvate, D-glucose, glutamate, glycine, or NADH. When indicated, specific inhibitors were added 15–30 min prior to L-lactate, glutamate, glycine, or NADH treatments. A detailed description of reagents and methodologies (cell culture, quantitative RT-PCR, Western blot, NAD/NADH assay, electrophysiology, calcium measurement, intracortical administration of compounds, and data analysis) used in this work can be found in [SI Materials and Methods](#).

**ACKNOWLEDGMENTS.** We thank Cendrine Barrière Borgioni, Elena Gasparotto, and Valérie Eligert for expert technical assistance; Romain Guiet for his help for calcium imaging quantification; and Sylvain Lengacher for helpful discussions. This work was supported by Swiss National Science Foundation Grants 31003A-130821/1 and 310030B-148169/1 and by the National Centre of Competence in Research (NCCR) Synapsy and the Biaggi and Panacée Foundations (P.J.M.).

- Suzuki A, et al. (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144(5):810–823.
- Gibbs ME, Anderson DG, Hertz L (2006) Inhibition of glycogenolysis in astrocytes interrupts memory consolidation in young chickens. *Glia* 54(3):214–222.
- Newman LA, Korol DL, Gold PE (2011) Lactate produced by glycogenolysis in astrocytes regulates memory processing. *PLoS ONE* 6(12):e28427.
- Alberini CM (2009) Transcription factors in long-term memory and synaptic plasticity. *Physiol Rev* 89(1):121–145.
- Bramham CR, et al. (2010) The Arc of synaptic memory. *Exp Brain Res* 200(2):125–140.
- Lynch MA (2004) Long-term potentiation and memory. *Physiol Rev* 84(1):87–136.
- Caroni P, Donato F, Muller D (2012) Structural plasticity upon learning: Regulation and functions. *Nat Rev Neurosci* 13(7):478–490.
- Zhou X, et al. (2009) N-methyl-D-aspartate-stimulated ERK1/2 signaling and the transcriptional up-regulation of plasticity-related genes are developmentally regulated following in vitro neuronal maturation. *J Neurosci Res* 87(12):2632–2644.
- Cammarota M, et al. (2000) Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: abolition by NMDA receptor blockade. *Brain Res Mol Brain Res* 76(1):36–46.
- Giovannini MG (2006) The role of the extracellular signal-regulated kinase pathway in memory encoding. *Rev Neurosci* 17(6):619–634.
- Sweatt JD (2001) The neuronal MAP kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* 76(1):1–10.
- Mullasseril P, et al. (2010) A subunit-selective potentiator of NR2C- and NR2D-containing NMDA receptors. *Nat Commun* 1:90.
- Cull-Candy SG, Leszkiewicz DN (2004) Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* 2004(255):re16.
- Hashimoto T, Hussien R, Oommen S, Gohil K, Brooks GA (2007) Lactate sensitive transcription factor network in L6 cells: Activation of MCT1 and mitochondrial biogenesis. *FASEB J* 21(10):2602–2612.
- Milovanova TN, et al. (2008) Lactate stimulates vasculogenic stem cells via the thio-redoxin system and engages an autocrine activation loop involving hypoxia-inducible factor 1. *Mol Cell Biol* 28(20):6248–6261.
- Zieker D, et al. (2008) Lactate modulates gene expression in human mesenchymal stem cells. *Langenbecks Arch Surg* 393(3):297–301.
- Formby B, Stern R (2003) Lactate-sensitive response elements in genes involved in hyaluronan catabolism. *Biochem Biophys Res Commun* 305(1):203–208.
- Zilberter Y, Zilberter T, Bregestovski P (2010) Neuronal activity in vitro and the in vivo reality: The role of energy homeostasis. *Trends Pharmacol Sci* 31(9):394–401.
- Lau CG, Zukin RS (2007) NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci* 8(6):413–426.
- Bergersen LH, Gjedde A (2012) Is lactate a volume transmitter of metabolic states of the brain? *Front Neuroenergetics* 4:5.
- Sullivan JM, et al. (1994) Identification of two cysteine residues that are required for redox modulation of the NMDA subtype of glutamate receptor. *Neuron* 13(4):929–936.
- Köhr G, Eckardt S, Lüddens H, Monyer H, Seeburg PH (1994) NMDA receptor channels: Subunit-specific potentiation by reducing agents. *Neuron* 12(5):1031–1040.
- Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: The revolution continues. *Nat Rev Neurosci* 6(8):626–640.
- Allaman I, Bélanger M, Magistretti PJ (2011) Astrocyte-neuron metabolic relationships: For better and for worse. *Trends Neurosci* 34(2):76–87.
- Bélanger M, Allaman I, Magistretti PJ (2011) Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metab* 14(6):724–738.
- Dringen R (2000) Metabolism and functions of glutathione in brain. *Prog Neurobiol* 62(6):649–671.
- Magistretti PJ (2006) Neuron-glia metabolic coupling and plasticity. *J Exp Biol* 209(Pt 12):2304–2311.
- Pellerin L, Magistretti PJ (1994) Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci USA* 91(22):10625–10629.
- Pellerin L, Magistretti PJ (2012) Sweet sixteen for ANLS. *J Cereb Blood Flow Metab* 32(7):1152–1166.
- Tsacopoulos M, Magistretti PJ (1996) Metabolic coupling between glia and neurons. *J Neurosci* 16(3):877–885.
- Choi HB, et al. (2012) Metabolic communication between astrocytes and neurons via bicarbonate-responsive soluble adenylyl cyclase. *Neuron* 75(6):1094–1104.
- Dringen R, Gebhardt R, Hamprecht B (1993) Glycogen in astrocytes: Possible function as lactate supply for neighboring cells. *Brain Res* 623(2):208–214.
- Magistretti PJ, Morrison JH (1988) Noradrenaline- and vasoactive intestinal peptide-containing neuronal systems in neocortex: Functional convergence with contrasting morphology. *Neuroscience* 24(2):367–378.
- Bozzo L, Puyal J, Chatton JY (2013) Lactate modulates the activity of primary cortical neurons through a receptor-mediated pathway. *PLoS ONE* 8(8):e71721.
- Tang F, et al. (2014) Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat Commun* 5:3284.